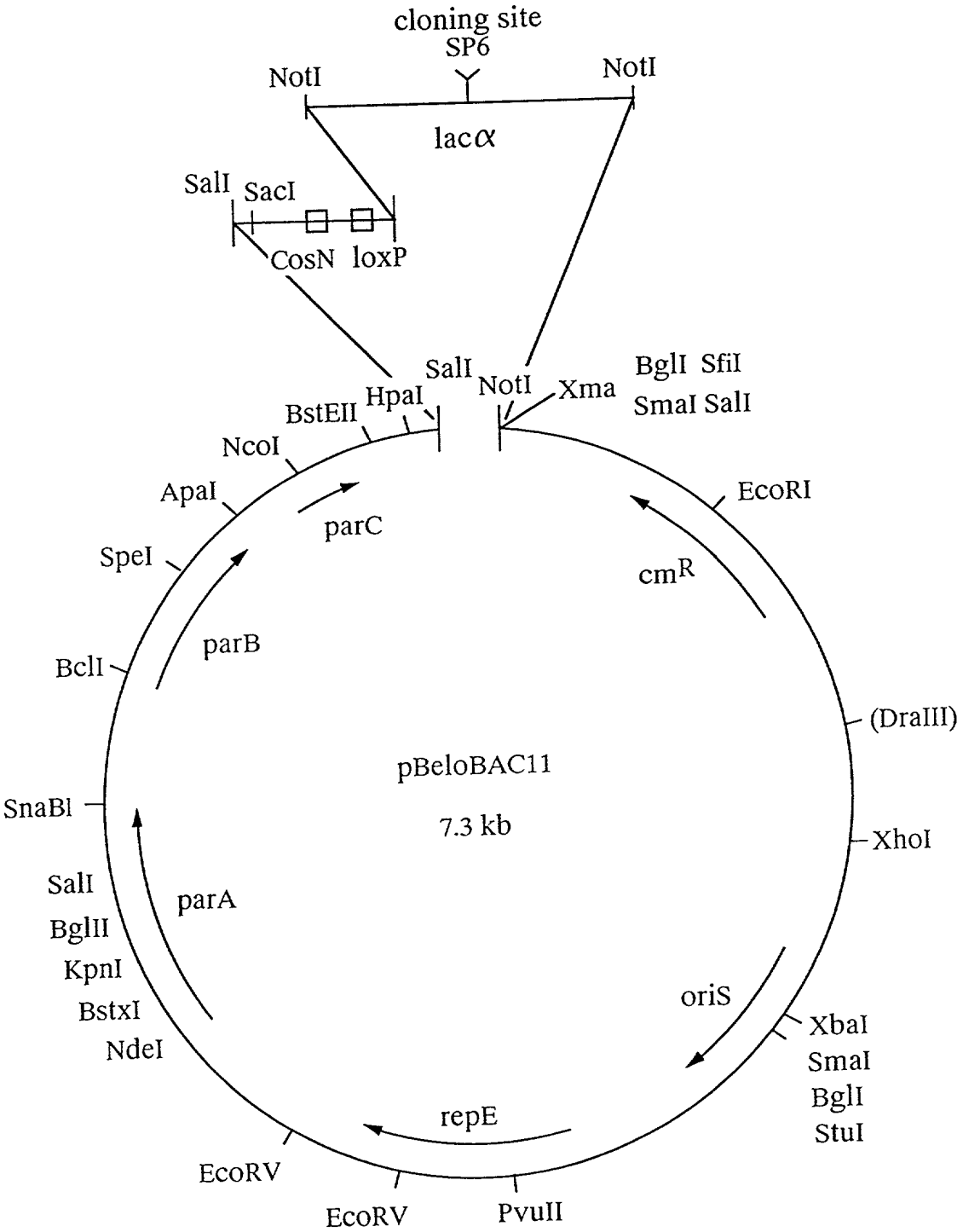
cloning site
SP6
NotI
NotI
lacα
SalI
SacI
CosN
loxP
SalI
NotI
Xma
BglI SfiI
SmaI SalI
BstEII
HpaI
NcoI
ApaI
SpeI
BclI
SnaBI
SalI
BglII
KpnI
BstXI
NdeI
EcoRV
EcoRV
PvuII
repE
oriS
XbaI
SmaI
BglI
StuI
XhoI
(DraIII)
EcoRI
cmR
parC
parB
parA
pBeloBAC11
7.3 kb

FIG. 1

Advantages of the BAC vector system

- Based on the F factor of *E. coli*
- Low level of chimeric clones
- Stable maintenance of heterologous DNA sequences
- Stable maintenance of large inserts
- Easy to isolate and manipulate BAC plasmids
- Efficient transformation system of host

Uses of BAC libraries and clones

- Physical characterization of genomes (i.e. cloning, mapping, FISH, contig assembly)
- Direct sequencing from BACs is possible
- Complementation of mutants *in vivo*
- Introduction of gene fusions into cells

FIG. 2

BAC libraries

Organism	Number of Clones	Average Insert Size (kb)
Sorghum	13,440	157
Rice	11,000	125
Bovine	23,040	146
Arabidopsis thaliana	3,948	100
Human	96,000	140
Magnaporthe grisea	4,128	66
Arabidopsis thaliana	9,000	60
Rice ssp. *japonica*	7,296	150
Rice ssp. *indica*	14,208	130
Human	96,000	110
Chicken	4,416	390
Lettuce	50,000	111
Barley	10,750	95
Magnaporthe grisea	9,216	130

FIG. 3

Comparison of different library types

vector type	insert size	90%*	99%*
plasmid	5 kb	2300	4600
cosmid	30 kb	382	765
BAC	136 kb	83	166

This is for a genome of 5 MB

* percent probability of library containing a specific sequence

FIG. 4

Expression of *Bacillus cereus* genes in *E. coli*

Phenotype tested	Positive clones detected ?
Ampicillin resistance	YES (3)
Casein hydrolysis	NO
Citrate utilization	NO
Esculin hydrolysis	YES (1)
Hemolysis	YES (1)
Lecithin hydrolysis	YES (2)
RecA function	NO
Starch hydrolysis	NO
Tween 80 hydrolysis	NO
Zwittermicin A resistance	YES (2)

FIG. 5

Heterologous expression of natural products pathways in *Streptomyces* species

Compound	Class	Producer	Expressed in	Size of DNA
nikkomycin	peptidyl nucleoside	*S. tendae*	*S. lividans*	31 plus 27 kb
puromycin	nucleoside	*S. alboniger*	*S. lividans* and *S. griseus*	13 kb
actinorhodin	polyketide	*S. coelicolor*	*S. parvulus*	~25 kb
cephamycin C	β-lactam	*S. cattleya*	*S. lividans*	29 kb
6-deoxy erythronolide B	polyketide	*Saccharopolyspora erythraea*	*S. coelicolor*	~25 kb

FIG. 6A

Heterologous expression of natural products pathways in *E. coli*

Compound	Class	Producer	Size of DNA	Comments
pyrrolnitrin	phenylpyrrole	*Pseudomonas fluorescens*	*<6.2 kb*	Tac promoter used
herbicolin A-like compound	depsi-glycopeptide	*Erwinia herbicola*		170 kb native plasmid conjugated
violacein	modified amino acid	*Chromobacterium violaceum*	*~15 kb*	also expressed in other Gram negative bacteria

FIG. 6B

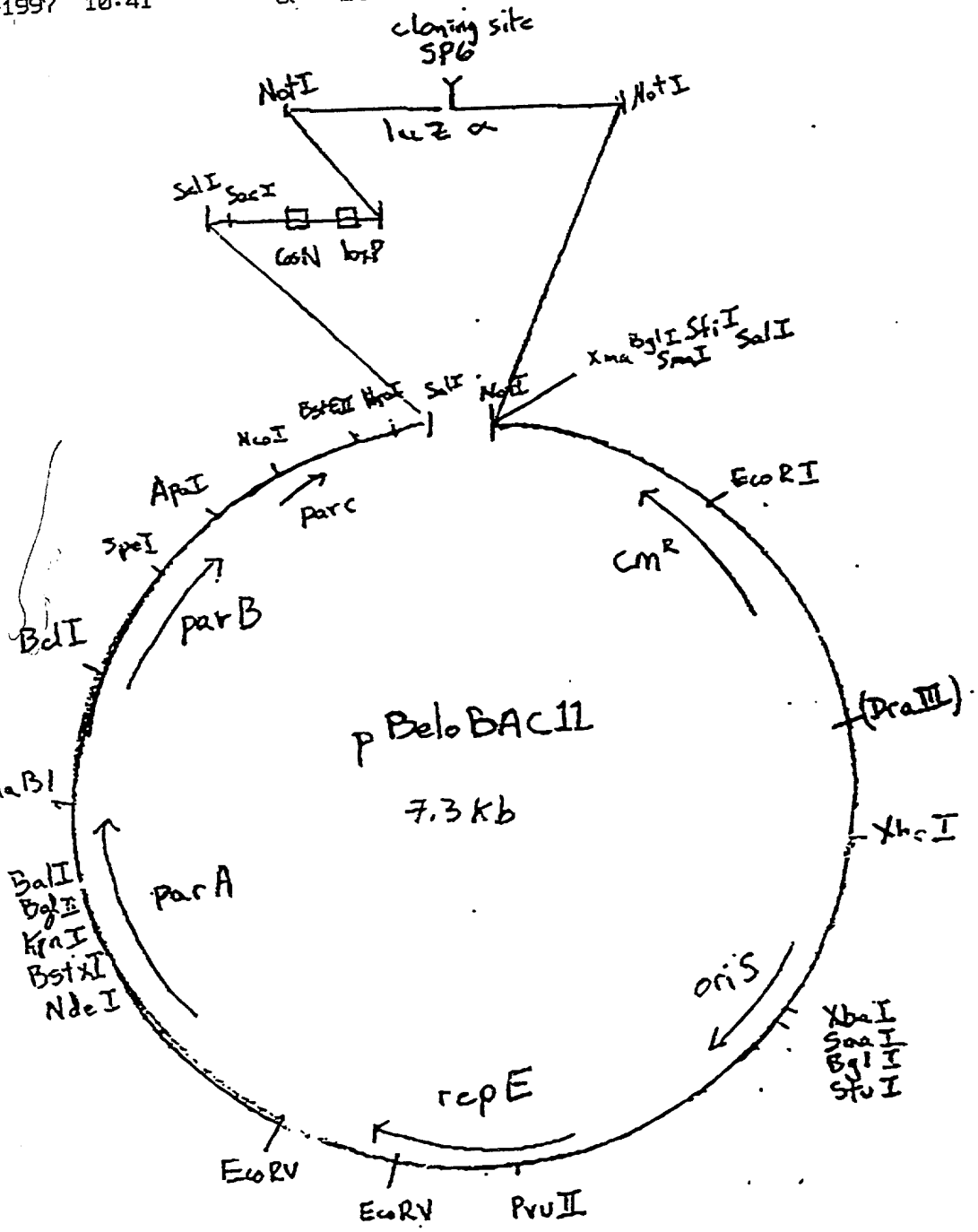

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Figure 2

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Figure 6

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Figure 6 (con't)

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